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*in kemijsko tehnologijo*

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*VABILO NA PREDAVANJE  
V OKVIRU DOKTORSKEGA ŠTUDIJA  
KEMIJSKE ZNANOSTI*

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z naslovom:

**Mass spectrometry in the research of  
environmentally relevant molecules: frog skin  
peptides, biomarkers, disinfection by-products,  
and emerging contaminants**

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*Vljudno vabljeni!*

## Abstract:

Modern mass spectrometry is the most powerful, sensitive, and informative tool for the analysis of structures and quantities of chemical compounds. It handles everything from isotopes of chemical elements to the most complex biopolymers. Although environmental services often use old general lists of several priority pollutants to estimate the level of contamination it is possible nowadays to get information about thousands of compounds in the environment. Tandem mass spectrometry and tandem chromatography, high resolution mass spectrometry allow resolving the most difficult cases, finding new disinfection by-products, novel contaminants and their transformation products, biomarkers of pollution and diseases. The studies of transformation of UV-filters, aqueous chlorination, establishing of the origin of the smell and pollution in Moscow, France, Chile, Arctic nicely support that statement.

The studies in the field of the diseases of the XXI Century make the investigators look at the living representatives of flora and fauna in order to understand their mechanisms of immune defense and protection from the deteriorating environment and pathogenic micro organisms. Amphibians as one of the leaders of immune resistance live on the Earth for hundreds of millions of years. Their dorsal glands produce a cocktail of biologically active compounds, mainly peptides, which may successfully fight micro organisms and even predators. Skin secretion of amphibians contains wide spectrum antibiotic and neuro peptides, critical for the immune response and active at the levels of  $10^{-9}$  M. They can also show antifungal and antiviral activities, stimulate insulin synthesis, inhibit NO synthesis, and be analgesics. The mechanism of action of antibiotic peptides is completely different in comparison to that of the existing pharmaceuticals: amphipathic  $\alpha$ -helix destroys phospholipid bilayer, leading to the lysis of the pathogenic cells. Since this mechanism prevents development of the pathogen's resistance, antimicrobial peptides are very perspective pharmaceuticals of future generations.

We obtain frog skin secretion by mild electric stimulation. LC-ESI-MS/MS analysis is carried out with ICR and Orbitrap mass spectrometers in CID, ECD, HCD, and ETD in MS<sup>2</sup> and MS<sup>3</sup> modes. The developed *de novo* sequencing algorithm involves the analysis of three versions of original samples of the frogs' skin secretion: intact, carboxamidomethylated and oxidized ones. The combined analysis allows achieving complete sequence coverage of all frog peptides including long (up to 50 aa) ones. It resolves the problems of S-S bonds, cyclization of short peptides, the presence of isobaric (e.g. lysine/glutamine) amino acid residues in the sequence. An efficient approach of easy and reliable differentiation between isomeric Leu/Ile involves production and isolation of primary z ions, followed by radical site initiation of their fragmentation with formation of w-ions, characteristic of the isomeric amino acid residues. Extracted ion plotting demonstrated its efficiency to detect all peptides related to a certain family. More than 200 new natural peptides were sequenced in terms of the present study. Their biological activity against microorganisms was studied. Thus, activity of brevinin 1Tb appeared to be in the nanomole range, i.e. that of the modern antibiotics.

Peptidome representation with 2D-maps based on the simple mass spectrometry parameters shows itself as a very convenient method to distinguish frogs of closely related species, and making mass spectrometry a powerful tool for taxonomy studies. The applicability of the proposed approach to differentiate the frogs of the same species but different populations (e.g. Russian and Slovenian) was shown. It involves changes in the sequences of similar peptides due to diversity of natural habitat. The animals face different microbes and synthesize the most efficient peptides to fight them. Therefore, interspecies and intraspecies biomarkers revealed by mass spectrometry may be very helpful for future taxonomy and biodiversity studies.